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The glycerol Biorefinery: Valorization of crude glycerol through its conversion into biofuels and green chemicals by mixed microbial consortia

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In recent years, the exponential growth of biodiesel production has led to concomitant increase in crude glycerol (CG). Even though pure glycerol represents a valuable product for the cosmetic and pharmaceutical industry, CG from biodiesel production is contaminated with compounds such as salts, methanol, soap, long chain fatty acids, etc. These contaminants cause high purification costs when converting glycerol by traditional chemico-physical methods. Thus, the use of anaerobic fermentation to convert abundant and low-priced glycerol streams generated in the production of biodiesel into higher value products has been proposed as a promising route to achieve economic viability in the biofuels industry [1]. However, most studies on glycerol fermentation so far have been focusing mainly on the use of purified glycerol and pure cultures, while typically observing reduced yields and/or productivities when testing CG[2].
The objective of this study was thus to select and adapt mixed microbial consortia (MMC) directly on CG, targeting MMC able to produce biofuels (hydrogen and ethanol) and/or green chemicals (i.e. VFAs and 1,3 propanediol). Various adaptation strategies were investigated for the enrichment of suitable and stable MMC, trying to overcome inhibition problems and enhance substrate degradation efficiency, as well as generation of soluble fermentation products. Moreover, different CG types were tested, including CG from animal fat derived (second-generation) biodiesel[2,3]. Repeated transfers in small batches and fed-batch conditions have been applied, comparing the use of different inocula (different origin and thermal pre-treatment), growth media and kinetic control, while continuous stirred tank reactor (CSTR) operations have been established to test the effect of pH and hydraulic retention time (HRT). Changes in microbial composition were monitored by means of Next Generation Sequencing technique, revealing a dominance of glycerol consuming species, such as *Clostridium*, *Klebsiella* and *Escherichia*[3]. Our previous experiments with biofuels production showed the possibility to reach a yield of > 0.9 mol $_{H2}$/mol $_{Gly}$ and 1 mol $_{EtOH}$/mol $_{Gly}$[4], in non-sterile conditions and without nutrient supplements[5]. We are now going to start a new investigation targeting the process intensification, by means of gas-stripping removal of ethanol, cell mass recirculation, as well as optimization of microaerophilic conditions to enhance ethanol production.
Regarding green chemicals, 1,3 propanediol turned out to be the dominant metabolite in most of the cases (maximum yield of 0.52 g/g), followed by butyrate. Maximum butyrate production yield reached around 0.4 g/g (with 10 g/L substrate), but stabilized at around 2 g/g during the steady state. Substrate (≈10 g/L) was completely consumed during CSTR experimentation (with only one exception), and no ethanol production was observed, while reaching a maximum of 0.46 g $_{EtOH}$/g in batch[3]. Interestingly, the same phenomenon was observed also with the MMC optimized for ethanol production. In general, different inoculum sources requested different approaches, but were able to be acclimatized to the diluted (also fat-derived) crude glycerol, without the need of any costly pretreatment of the feed. Ongoing work is now focusing on identification of the operating parameters for maintaining a stable MMC and statistical optimization of key parameters for enhanced green chemicals production.

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